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Original article

Preliminary survey of indigenous parasites associated with *Phyllocnistis citrella* Stainton (Lepidoptera, Gracillariidae) in Jeju, Korea

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ABSTRACT

In order to identify the parasitoids of *Phyllocnistis citrella* Stainton [commonly known as the citrus leafminer (CLM)], we collected CLM larvae and pupae in the Seogwipo area of Jeju-do, identified parasitoid species, and determined parasitism rates. In May 2014, nine parasitoids were identified from the CLM larvae and pupae that infested 300 fresh citrus leaves, yielding a parasitism rate of 3.03%. In August 2014, 53 parasitoid wasps emerged from 203 collected CLM larvae and pupae, indicating that the parasitism rate rose to 25.84%. The parasitoid wasps observed in May 2014 consisted of five individuals of *Sympiesis* sp. 1 (Eulophidae), one *Pnigalio* sp. 1 (Eulophidae), and three *Aphelinus* sp. 1 (Aphelinidae). The highest proportion of the parasitic wasps that emerged in August 2014 were *Sympiesis* sp. 1 ($n = 49$), followed by *Quadrastichus* sp. 1 (Eulophidae; $n = 4$). This study documents the seasonal parasitism rate of parasitic wasps and basic characteristics of each parasitoid in Korea.

Introduction

Phyllocnistis citrella Stainton (Lepidoptera: Phyllocnistidae), also known as the citrus leafminer (CLM), is a very small moth and is a pest of citrus plants. There is only one known species in Korea (Byun et al 2009). It is native to Southern Asia, but within the past 5 years has spread to almost all citrus growing areas worldwide (e.g. Commonwealth Agricultural Bureau International 1995; Hoy and Nguyen 1997). It spreads rapidly. For example, it entered Spain in the summer of 1993, spread across the country within 1 year, and was found along the Mediterranean coast by 1995 (Urbaneja et al 2000).

The CLM is a pest commonly known to citrus growers. It deposits eggs on citrus leaves and the larvae grow beneath the fragile ventral surface of fresh leaves, impeding their growth and thus directly affecting harvest. Although biological pest control is the best economic and ecological option to combat this pest, South Korea still relies on regular chemical control because of a lack of research regarding efficient natural enemies such as parasitoids and predators. Biological control of the CLM is a promising control method for population suppression through the early settlement stage of natural enemies. According to recent data, about 80 species of CLM parasitoids have been detected worldwide (Schauff et al 1998; Kernasa et al 2008). In the New World, over 20 additional species have been found because of their continuing spread over wider geographic areas, and intensive research on these parasitoids is ongoing (Legaspi et al 2001). The CLM was first discovered in Florida, USA in 1993, and it soon became a matter of great concern by rapidly colonizing citrus-growing areas extending over 270,000 ha. In 1994, by introducing its parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae) from Australia to Florida, the CLM pest was successfully controlled (Hoy and Nguyen 1997; Argov and Rössler 1998). Methods for indoor rearing and breeding of CLM parasitoids were reported by Smith and Hoy (1995).

No serious harvest-related CLM damage has been reported in Korea. Nevertheless, given the increasing damage cases in other countries, as well as climate and environmental changes, the CLM could become an economic pest causing serious harvest damage in Korea as well. For citrus growers relying on year-round operation of

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their greenhouses for citrus production, there is a need to explore various CLM control measures. The CLM caused enormous damage to the citrus production in Spain and the USA 2 decades ago, but it was demonstrated on those occasions that successful parasitoid-based biological pest control is possible (Urbaneja et al 2000; Lim and Hoy 2005; Lim et al 2006). Thus, it is expected that CLM-related damage will be minimized by developing biological control methods using parasitoids. For this reason, this study was conducted to secure basic data to establish biological CLM control measures in Korea. To this end, we explored indigenous parasitoids in the areas invaded by the CLM and selected promising species that could be used as a viable option for combating the CLM by developing methods for indoor mass rearing.

Materials and methods

We obtained CLM larvae and pupae from the Citrus Research Institute, National Institute of Horticultural and Herbal Science, Rural Development Administration located in Seoguipo, Jeju-do, Korea (Figure 1). As the first step to identify parasitoids of CLM larvae and pupae, we collected larvae and pupae from citrus leaves in May 2014 and August 2014. We put each collected individual in a plastic bag, and documented the emergence of parasitoids and parasitism rates on the CLM. Next, we collected 170 larvae and pupae for 2 days (August 21, 2014 and August 22, 2014) from insecticide-treated and nontreated host trees, and investigated intergroup differences in CLM mortality and parasitism (Figure 2). This study was performed in the citrus orchard at the Citrus Research Institute, a citrus farm near the Institute, and the experimental citrus plots within the Institute. The parasitoid wasps were examined with a stereomicroscope (M205 A; Leica, Wetzlar, Germany).

Results and discussion

Bionomics of the CLM

Individual infested leaves collected outdoors were kept separately to observe the complete metamorphosis and developmental features of the CLM by stage. The larvae form short tunnels immediately after hatching, feeding on mesophylls and sucking phloem sap in small amounts. The spot where the egg hatched turns black. After reaching a certain size, larvae start to form shallow, long tunnels, which leave dark brown lines. They are bright green, but when they pupate, their color changes to ash brown from the head down to the wings and light brown in the abdominal area. Adults have long antennae and ash white forewings with one black spot on each wing tip. Citrus leaves mined by the CLM dry out eventually because of a lack of chlorophyll, which reduces the esthetic value of the trees and causes harvest damage (Figure 3).

Parasitoids and parasitism ratio

The parasitoids of the CLM were emerged and identified as *Sympiesis* sp. 1, *Quadratstichus* sp. 1 and *Pnigalio* sp.1 of Eulophidae, and *Aphelinus* sp.1 of Aphelinidae (Figures 4, 5). They all appear to be solitary parasitoids, especially *Sympiesis* sp. 1 (Figure 5). Despite a wide variety of the CLM parasitoids, general preference has focused on third instar larvae, with the parasitism rate increasing across the seasons, reaching 70% by summer in Spain (Urbaneja et al 2000). Accordingly, a substantial increase in the CLM parasitism rate was observed from 3.0% in May 2014 to 25.84% in August 2014 in the Citrus Research Institute in Seogwipo, Jeju-do, especially with *Sympiesis* sp. 1 demonstrating a high parasitism rate (Table 1).

Figure 1. Sampling areas at the Citrus Research Institute on Jeju Island: A, Entry of Citrus Research Institute; B, citrus farm around the Citrus Research Institute; and C, experimental citrus plot in citrus research institute.

Figure 2. (A,B) Vinyl collection bags and (C) parasitoid emerged from the citrus leafminer.

Figure 3. Life history of the citrus leafminer: A, egg; B, larva; C, pupa; D, adult; and E, infected leaf.

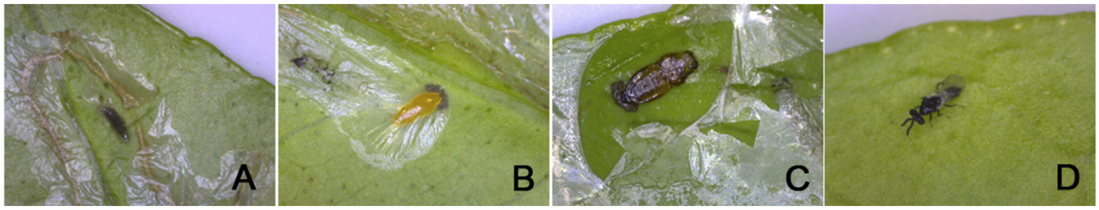

Figure 4. Emergence of a parasitoid of the citrus leafminer. A–D: young larva to adult.

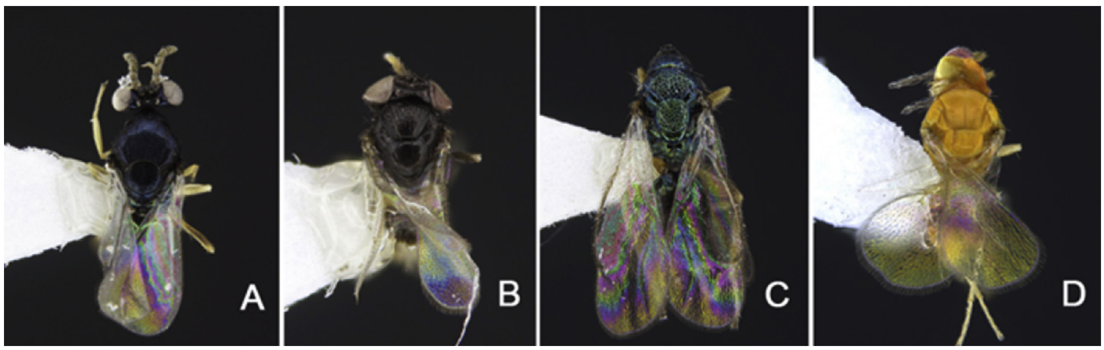

Figure 5. Parasitoid of the citrus leafminer on Jeju Island: A, *Sympiesis* sp. 1; B, *Aphelinus* sp. 1; C, *Pnigalio* sp. 1; D, *Quadrastichus* sp. 1.

Table 1. Parasitoid list of the citrus leafminer on Jeju Island during May 2014 and August 2014.

Date (No. of samples)	Parasitoids					Parasitism (%)
	Family	Species	♀	♂	Total	
May 2014 (300)	Eulophidae	*Sympiesis* sp. 1	4	1	5	1.67
		Pnigalio sp. 1	1		1	0.33
	Aphelinidae	*Aphelinus* sp. 1	2	1	3	1.0
21 Aug 2014 (203)	Eulophidae	*Sympiesis* sp. 1	19	30	49	24.1
		Quadrastichus sp. 1	1	3	4	1.74

Mortality and parasitism

We investigated differences in CLM mortality and parasitism among the samples collected from the citrus orchard at the entrance of the Citrus Research Institute (Group O), the citrus farm near the Citrus Research Institute (Group F), insecticide-treated experimental plot (Group I), and insecticide nontreated experimental plot (Group N) at the Citrus Research Station (Table 2). Although the CLM mortality and parasitism of Group I were respectively 96.0% and 4.0%, those of Group N were 50.0% and 42.9%, demonstrating substantial differences in both mortality and parasitism.

In particular, Group N exhibited a higher parasitism rate than Group F (42.9% vs. 26.9%). Although the direct effects of insecticide on parasitism rate could not be derived from this result, it appeared that as CLM density rose and application of insecticide declined, parasitoid density increased. This relationship needs to be investigated in detail in future research. The findings of this study highlight the need for research to develop methods of indoor breeding for the main parasitoids of the CLM, such as *Sympiesis* sp. 1 (Eulophidae), in order to use them as biological control agents.

This study was conducted with the goal of investigating parasitoid wasps to explore their potential as biological control agents to combat the spread of the CLM, which causes damage to citrus harvest. Although this study was significant in that it verified the plausibility of biological control of the CLM using parasitoids, it illustrated the limitations in securing sufficient data because of the short study period and limited sampling areas. The study results will serve as basic data for follow-up research that could focus on obtaining necessary data to enable practical application of biological control of the CLM by expanding the survey areas and scope of parasitoid species and variants.

Table 2. Mortality, emergence rate, and parasitism of the citrus leafminer at the Citrus Research Station and the citrus farm during August 21–22, 2014.

Site	Stage	Number of collection	Mortality of the leafminer (%)	Emergence rate of the leafminer (%)	Parasitism (%)
Citrus Research Station[a]	Last instar larva	42	42.9	33.3	23.8
	Pupa	24	37.5	58.3	4.2
Citrus farm[b]	Last instar larva	26	53.8	19.2	26.9
	Pupa	15	60.0	40.0	0
Noninsecticide treatment[c]	Last instar larva	14	50.0	7.1	42.9
	Pupa	15	26.7	73.3	0
Insecticide treatment[d]	Last instar larva	25	96.0	0	4.0
	Pupa	9	44.4	44.4	11.1

[a] Entry of Citrus Research Station (Group O).
[b] Citrus farm around the Citrus Research Station (Group F).
[c] Experimental citrus plot in Citrus Research Station, noninsecticide treated (Group N).
[d] Experimental citrus plot in Citrus Research Station, insecticide treated (Group I).

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References

Argov Y, Rössler Y. 1998. Rearing methods for the citrus leafminer *Phyllocnistis citrella* Stainton and its parasitoids in Israel. *Biological Control* 11:18–21.

Byun BK, Park KT, Bae YS, et al. 2009. *A checklist of the Microlepidoptera in Korea (Lepidoptera), Korea National Arboretum*. Seoul: SAMSUNGAD.COM. p. 413.

Commonwealth Agricultural Bureau International (CABI). 1995. *Distribution maps of pests*. Series A: Map No. 274 (2nd revision).

Hoy MA, Nguyen R. 1997. Classical biological control of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae): Theory, practice, art and Science. *Tropical Lepidoptera* 8 (Suppl. 1):1–19.

Kernasa O, Suasa-ard W, Kosol Charernsom K. 2008. Citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) and its natural enemies. *Kasetsart Journal: Natural Science* 42:238–245.

Legaspi JC, French JV, Zuniga AG, Legaspi BC. 2001. Population dynamics of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), and its natural enemies in Texas and Mexico. *Biological Control* 21:84–90.

Lim UT, Hoy MA. 2005. Biological assessment in quarantine of *Semielacher petiolatus* (Hymenoptera: Eulophidae) as a potential classical biological control agent of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), in Florida. *Biological Control* 33:87–95.

Lim UT, Zappalà L, Hoy MA. 2006. Pre-release evaluation of *Semielacher petiolatus* (Hymenoptera: Eulophidae) in quarantine for the control of citrus leafminer: host discrimination, relative humidity tolerance, and alternative hosts. *Biological Control* 36:65–73.

Schauff ME, LaSalle J, Wijesekara GA. 1998. The genera of chalcid parasites (Hymenoptera: Chalcidoidea) of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). *Journal of Natural History* 32:1001–1056.

Smith JM, Hoy MA. 1995. Rearing methods for *Ageniaspis citricola* (Hymenoptera: Encyrtidae) and *Cirrospilus quadristriatus* (Hymenoptera: Eulophidae) released in a classical biological control program for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). *Florida Entomologist* 78:600–608.

Urbaneja A, Llácer E, Tomás O, et al. 2000. Indigenous natural enemies associated with *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in eastern Spain. *Biological Control* 18:199–207.